potential seemed to overlap with skeletal development in spatiotemporal manner. In addition to these histological observations, we analyzed the expression patters of Gdf5, Runx2, Osterix *X. tropicalis* homologues by *in situ* hybridization throughout metamorphosis. We will report the spatiotemporal correlation between the decline of the regeneration potential and the expression pattern of these genes essential for chondrogenesis and osteogenesis.

ANALYSIS OF THE MOLECULAR MECHANISM OF PROLIFERATING CELL INDUCTION IN REGENERATING *XENOPUS LAEVIS* TADPOLE TAILS

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In the regenerating animal organs, proliferating cells that will form the regenerating tissues appear after the wound healing and the wound epidermis covering the stump plays an important role for the appearance of those proliferating cells. To elucidate the molecular basis which links wound epidermis covering to the appearance of proliferating cells, we searched for genes whose expression change during this period, using regenerating *Xenopus laevis* tadpole tails. So far, we have identified 6 genes whose expressions are up-regulated just before the appearance of proliferating cells. The functions of these 6 candidate genes have not been reported. In contrast, no significant change in the expression of these genes was observed in the amputated tails of tadpoles of "refractory period", when tadpoles lose their regeneration ability. These results suggest that these genes are probably involved in the process specific to the regeneration.

IMCOMPLETE ANTERIOR-POSTERIOR AXIS FORMATION IN *XENOPUS* FROGLET LIMB REGENERATION

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While urodele amphibians can regenerate their appendages perfectly, a *Xenopus* froglet, a young adult after metamorphosis, merely forms a cartilaginous spike structure on its amputated limb mostly because of deficient ability for pattern formation in the redevelopment phase. Pattern in the limb can be divided into morphology along orthogonal three axes; the anterior-posterior, the proximal-distal, and the dorsal-ventral axes, and we focused on the anterior-posterior axis formation in the froglet limb regeneration. By RT-PCR and in situ hybridization on sections, we found that expression of some genes in *sonic hedgehog* (*shh*) signaling cascade, including *shh* itself, is not detected during the early stage of froglet limb regeneration. These results suggest that the defect of *shh* signaling is one of reasons for the pattern-deficient regeneration in *Xenopus* froglet. Upstream mechanism on the defect of *shh* re-expression and the possible improvement of the pattern formation in the froglet limb regeneration will be discussed.

LOSS OF REGENERATION CAPACITY IN THE OPTIC TECTUM OF *XENOPUS*

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It is widely known in the anuran amphibians that they can regenerate many organs only during larval life. In the present study, we have confirmed that the optic tectum in the midbrain regenerates in larvae but not in adult of Xenopus laevis, like the case in the telencephalon. A good corelation was observed in the proliferation of the ependymal cells and the capacity for regenarating. Decresed proliferation was also evident even after the partial removal of the tectum, dislike the case in telencephalon, suggesting that the cause of loss of regeneration capacity might vary depending on the part of the Xenopus brain.

THE APOPTOSIS OF OVULATED OOCYTES OF THE JAPANESE MEDAKA *ORYZIAS LATIPES*

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Without fertilization, highly synchronous apoptosis of starfish eggs starts 10 h after germinal vesicle breakdown, which varies according to season and individual animals. Apoptosis of mouse and fish (sea bream) oocytes has also been reported. It is, however, still uncertain whether apoptosis occurs in unfertilized eggs of many other animals. Since eggs of Japanese medaka fish are large and transparent, apoptotic events such as membrane blebbing, shrinkage and apoptotic body formation, may be easily observed. Indeed, 40 h after ovulation, 90% unfertilized eggs showed shrinkage, suggesting that apoptosis occurred in unfertilized eggs.

It is well known that caspase-3 is the key enzyme to execute apoptosis. When homogenized eggs were assayed to detect caspase-3 like DEVDase activity, the activity increased significantly in the preparations from eggs showing shrinkage. These results indicate that apoptosis of medaka eggs starts around 40 h after ovulation.

MECHANISM OF INHIBITION OF CASPASE-3 ACTIVITY IN STARFISH UNFERTILIZED EGGS

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Meiosis of immature starfish oocytes are reinitiated by 1-methyl-adenine (1-MA), which is released from follicle cells, causing germinal vesicle breakdown (GVBD). If not fertilized, they complete both meiotic divisions to yield haploid interphase-arrested eggs. Although starfish immature oocytes can live more than 1wk in seawater, postmeiotic eggs synchronously and rapidly undergo apoptosis within 24 h after 1-MA treatment. Usually, membrane blebbing starts 8-12h after 1-MA treatment and is followed by apoptotic body formation. The timing of apoptosis depends on the animals and seasons, but apoptotic processes are highly synchronous in the same animal. We have revealed that caspase-3 activity is responsible for egg apoptosis. When human active caspase-3 was injected into immature oocytes or eggs 3 hours after 1-MA treatment, caspase-3 activity decreased rapidly and abolished completely within 2 hours. Also, degradation of injected caspase-3 occurred within 2 hours. Proteasome inhibitor blocked the degradation of this enzyme. These results suggest that caspase-3 inhibition is due to proteasome-mediated degradation of caspase-3 in young eggs.

THE CELL DEATH OF *XENOPUS* PERMANENT BLASTULA-TYPE EMBRYOS

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Ablation of vegetal cytoplasm *Xenopus* eggs results in two distinct types of axis lacking embryos. When the deleted volume was 20-40% (relative surface area), the embryos underwent ventral-type gastrulation and formed ventral mesodermal tissues. When the deleted volume was more than 60%, the embryo did not gastrulate nor make mesodermal structures. We have designated these two types of embryos as gastrulating nonaxial embryos (GNEs) and permanent blastula-type embryos (PBEs), respectively. the development of permanent blastula-type embryos (PBEs) consisted of atypical epidermis. Although GNEs survived more than three weeks at room temperature, PBEs degenerated at 8 days of culture (stage 45). Caspase activity assay revealed that PBEs underwent apoptosis at stages 20-45. We reasoned that the absence of endomesoderm in PBEs should cause the observed apoptosis. We injected 15 pg/blastomere *VegT*, an endomesodremal determinant in *Xenopus*. The injected PBEs formed a blastopore and survived more than 3 weeks.

ASYNCHRONOUS PROGRESSION OF PROGRAMMED CELL DEATH ENSURES THE EPITHELIAL STRUCTURE DURING LARVAL ARM RESORPTION IN SEA URCHIN METAMORPHOSIS

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When the competent larva of sea urchin is experimentally induced metamorphosis, its arms resorb without any morphological collapse as an organ. Quantitative analysis was ultrastructurally conducted to ascertain the relationship between ongoing pattern of programmed cell death (PCD) and disorganization on septate junction (SJ) in two kinds of the constituent epithelial cells (ECs) of the arm. In the columnar ECs that form the ciliary band, apoptosis initiated synchronously to occur among them. Its terminal phase, however, was around differently from the cells to cells. The SJ tended to remain among the cells until the terminal phase of apoptosis. The squamous ECs, which are distributed near the ciliary bands, underwent PCD seemingly distinct from apoptosis. Its timing to initiate was not only asynchronously among them but also delayed as compared to the columnar ECs. Any alterations of the SJ could not to be detected in spite of dramatic progression of PCD. These data suggests that at least two of mechanisms underlay larval arm resorption during metamorphosis; those are that PCD progresses asynchronously among the ECs, and that SJ is prolonged its fate during ongoing of PCD.

PROGRAMMED CELL DEATH IN THE *BOMBYX* FAT BODY

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In most insects, fat body cells survive through the larval and pupal period with undergoing cellular reconstruction at pupal metamorphosis and transformation into adult fat body cells. By contrast, larval fat body in some Diptera and Hymenoptera undergoes programmed cell death (PCD) at metamorphosis and is replaced by adult fat body, which aries from imaginal fat body cells. Here, we show that *Bombyx* fat body undergoes PCD at the larval-pupal metamorphosis. The fat body exhibited nuclear fragmentation and DNA fragmentation, which are characteristic of the PCD, from 1 to 2 days after pupation. Simultaneously, caspase-3 like activity was increased. When day 6 fifth instar fat bodies were cultured with 2 μ M 20E, they underwent PCD, while an addition of hemolymph of day 5 fifth instar larvae to the culture suppressed the PCD. Those data suggest that the PCD is regulated by not only hormones but also a factor in hemolymph.